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Age matters: collagen birefringence of superficial articular cartilage is increased in young guinea-pigs but decreased in older animals after identical physiological type of joint loading

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Summary

Objective: To compare responses of the collagen network and glycosaminoglycans (GAGs) of articular cartilage to physiological type of joint loading in young growing and adult mature guinea-pigs.

Design: 10- and 44-week-old guinea-pigs were accustomed to treadmill running for 3 weeks. Thereafter the animals ran 2500 m/day, 5 days a week, for 15 weeks. Articular cartilage specimens from knee joints were collected at 28 and 62 weeks. Osteoarthritis (OA) prevalence and severity was evaluated by aid of light microscopy. The degree of collagen fibril network organization and content was analyzed with quantitative polarized light microscopy. The local concentration of GAGs was determined from cartilage sections with digital densitometry after safranin-O staining.

Results: In the young guinea-pigs, running increased up to 24% the optical retardation of polarized light by collagen in the superficial articular cartilage of femur, indicating either a higher degree of fibril assembly and organization or increased amount of collagen, or both. In contrast, in the adult mature animals the optical retardation decreased almost 50% after joint loading (P<0.01–0.001). Running did not increase cartilage fibrillation. Significant changes in GAG content of cartilage were not found either in the young or adult mature runners.

Conclusions: Increased birefringence of the superficial articular cartilage after joint loading in young guinea-pigs can be interpreted to be a sign of improved and decreased birefringence in older animals a sign of worsened property of the collagen network. It can be suggested therefore that joint loading strengthened the collagen network in the young runners. It can be hypothesized further that with time the inferior property of the collagen network predisposes the older runners to earlier OA than in controls. © 2001 OsteoArthritis Research Society International

Key words: Collagen, Joint loading, Osteoarthritis, Proteoglycans, Guinea-pig.

Introduction

Joint loading and movement contribute to improvement of the biological properties of articular cartilage, such as proteoglycan content and stiffness of cartilage[1]. Organization of the type II, IX, and XI collagens[2,3] and distribution of the glycosaminoglycans (GAGs) within the extracellular matrix[4] allow articular cartilage to withstand and redistribute stresses and strains applied on joint surfaces. GAGs are responsible for the elasticity and resilience of cartilage[5], while collagens provide the tensile stiffness and strength for the tissue[6–9]. Ultrastructurally, the collagen network shows characteristic structural patterns in the three zones of uncalcified articular cartilage[10,11]. In the superficial zone, collagen fibrils run parallel to the cartilage surface. In the intermediate zone, they curve and fan out randomly into the depth of tissue and finally, in the deep zone, the fibrils run perpendicularly to the surface. Mechanisms, which would explain reactions of the resident chondrocytes to stress during joint loading are unclear[12]. Analyses from different zones of articular cartilage have shown an increasing gradient of PGs from the superficial zone to the deep cartilage[13]. An identical zonal gradient of PG-synthesis and catabolism by residing chondrocytes has been shown in cell and tissue culture conditions with [^{35}S]labeling[14,15]. These experiments have also shown an age-related decrease in the [^{35}S]sulfate incorporation by chondrocytes.

In early spontaneous osteoarthritis (OA), enhanced synthesis of type II collagen[16–19] and GAGs[20,21] have been observed. In more advanced OA, a decreased synthesis of matrix GAGs[22,23] and increased collagen fibril damage was reported[24,25].

In Dunkin-Hartley guinea-pigs, the medial compartment of the knee joint is prone to spontaneous, progressive articular cartilage degeneration with aging. The lesions resemble those of human OA[26,27]. In these animals, scorbutic diet and excessive body weight accelerate cartilage degeneration significantly[28,29]. Superficial knee cartilage fibrillation has been reported as early as at 12 weeks

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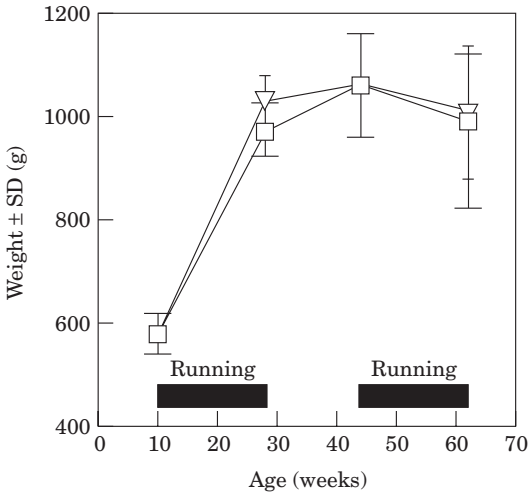

Fig. 1. Weight development of guinea-pigs during the experiment (mean±S.D.; g). Horizontal bars (length 18 weeks) indicate the initial 3-week training and the following 15-week running exercise period (distance 2500 m/day, 5 days a week, speed 0.33 m/s). No significant difference was observed between the runner (—▽—) and control (—□—) groups (Mann–Whitney U test).

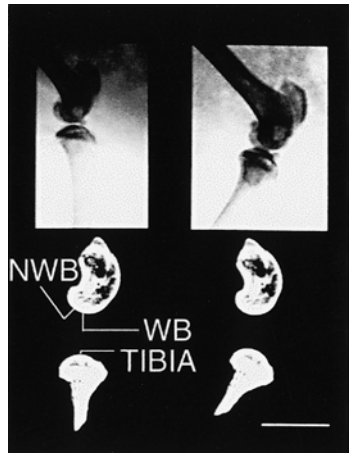

Fig. 2. Roentgenographic determination of the weightbearing (WB) and non-weightbearing (NWB) areas of femur and the weightbearing tibia (TIBIA). X-rays were taken at 20° and 80° angles from flexed knee joints of anaesthetized animals. The angles were estimated from digitized video recordings during normal running cycle at a speed of 0.33 m/s. In the figure, the sagittally oriented medial femoral condyle and its tibial counterpart are illustrated. Scale bar=1 cm.

of age, and at 50 weeks the majority of male guinea-pigs exhibit mild to moderate degeneration extending to the deep zones. The medial tibial plateau, uncovered by the meniscus, is usually the first and the most severely affected site[30]. The femoral condyles are nearly equally frequently fibrillated but the erosive lesions appear to be limited to the superficial articular cartilage[30]. Simultaneously, the OA-related 3-B-3-antigenicity appears in the knee joint cartilage but, interestingly, not in the hip joint[31]. There is also evidence that in Dunkin-Hartley guinea-pigs the biosynthesis of sulfated PGs is markedly accelerated at an early stage of surgically induced OA[32].

The responses of the cartilage collagen network in young growing or adult mature guinea-pigs to joint loading are not known. It is not known either how an increased joint loading influences the incidence and severity of spontaneously occurring OA in guinea-pigs. Therefore, in this study, we aimed at investigating how a physiological type of joint loading, i.e. running exercise, modulates the articular cartilage collagen network and proteoglycans in young growing and adult mature guinea-pigs and affects the course and severity of spontaneously occurring OA. Responses of the fibrillar collagen network and GAGs were quantitated with digital microscopy from different zones of articular cartilage. OA lesions were scored from histological sections by light microscopy.

Materials and methods

Ninety female Dunkin-Hartley guinea-pigs (Møllegaard Breeding, Ejby, Denmark) were housed under standardized conditions in plastic cages with floor dimensions of 0.42 m×0.52 m, three animals per cage. Water, commercial guinea-pig food pellets (Hankkija, Kolppi, Finland) and fresh cabbage for ascorbic acid supplementation were freely available. Lights on–lights off cycle was 12/12 h. The design of the experiment is shown in Fig. 1. The animals were randomly divided into two running groups: *young growing* 10-week-old (*N*=15), and *adult mature* 44-week-old guinea-pigs (*N*=14). They were accustomed to treadmill running for 3 weeks. After that they ran 2500 m/day for 15 weeks (5 days a week) at the speed of 0.33 m/s. Cartilage specimens were collected from the animals at 28 weeks (*N*=15) and at 62 (*N*=15) weeks. Tissues from control animals were collected at the age of 10 weeks (*N*=15), 28 weeks (*N*=15), 44 weeks (*N*=14) and 62 weeks (*N*=13). The Animal Care and Use Committee of the University of Kuopio approved the design of the experiment.

DETERMINATION OF THE WEIGHTBEARING AND NON-WEIGHTBEARING AREAS OF THE FEMORAL AND TIBIAL CONDYLES

The tibiofemoral contact area and the weightbearing (WB) and non-weightbearing areas (NWB) of the medial femoral condyle and the weightbearing tibia (TIBIA) were determined using digitized video recordings and roentgenograms (for details, see legend for Fig. 2).

SPECIMEN PREPARATION

The guinea-pigs were sacrificed with an intraperitoneal overdose of sodium pentothal. Frontal sections were prepared from femur and tibia. A 3-mm-thick slab was sawn antero-posteriorly and freed from the medial condyle of femur (Figs 2 and 3). The slab was fixed in neutral phosphate buffered formalin, decalcified in 10% EDTA, dehydrated in ascending alcohols and embedded in Paraplast Plus (Sherwood Medical, St Louis, MO, U.S.A.) as previously described[33]. The horizontally embedded condyle was put under a stereomicroscope and by perpendicular cuts with a disposable microtome blade, the WB, NWB and TIBIA areas were separated from the tissue block (Fig. 2). The blocks were re-embedded and the final section plane, perpendicular to the cartilage surface, ran mediolaterally through the condyle. Three-μm-thick sections for safranin-O staining and 7-μm-thick sections for polarized microscopy were cut with an LKB Historange

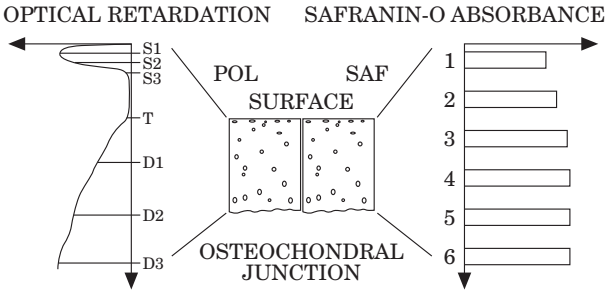

Fig. 3. Preparation of histological sections for polarized light microscopy (POL) and safranin-O staining (SAF). The section plane was perpendicular to the articular cartilage surface. Measurements were made from the surface to the osteochondral junction. In POL, optical retardation profile from the superficial and deep collagen network was divided into three classes denoted S1–S3 and D1–D3, respectively. In S1–S3 the course of the fibrils was mainly parallel while in D1–D3 the fibrils ran perpendicularly to the cartilage surface. (T) denotes the thickness of the non-birefringent transition layer of collagen fibrils where the parallel course changes to perpendicular. In safranin-O stained sections the stain absorbance profile was divided into six classes along with the cartilage depth.

rotary microtome (LKB, Bromma, Sweden). The sections were blind coded and the further processing of the control and runner specimens was made simultaneously using the same reagents and stain solutions.

GRADING OF OSTEOARTHRITIC LESIONS

The incidence and severity of osteoarthritic lesions was scored from 3-μm-thick safranin-O stained sections[33]. Since most of the lesions extended histologically only to the intermediate zone and were relatively mild, we adopted the scoring system previously used with mice[34]. The scoring system is explained in Fig. 4.

QUANTITATIVE POLARIZED LIGHT MICROSCOPY OF COLLAGEN

The degree of submicroscopic organization and the amount of fibrillar collagen were estimated with quantitative polarized light microscopy (PLM) of unstained histological sections. Optical retardation of plane polarized light due to the collagen fibrils of articular cartilage was quantitated with digital image-analysis as previously described[35]. Briefly, 7-μm-thick sections were carefully dewaxed in xylene and rehydrated in alcohols. Tissue GAGs were digested with testicular hyaluronidase and after dehydration in ascending alcohols the sections were mounted with DPX (Difco, East Molesey, U.K.)[35]. The unstained sections were examined with a Leitz Ortholux II microscope equipped with crossed polarizer and analyser, strain-free optics and a 16×/0.45 N.A Fluotar objective (Leitz, Wetzlar, Germany) and an interference monochromator (λ=595±10 nm, Optometrics Inc., Ayer, MA, U.S.A.). A Photometrics CH 250 camera and image-analysis system were calibrated to optical retardation units according to previously reported method[35].

Absolute thickness of articular cartilage (μm) from cartilage surface to osteochondral junction was determined and the mode distribution profile of the optical retardation was generated between these landmarks using a 318-μm wide measuring region (Fig. 3). From the retardation profile the thickness of the parallelly, obliquely, and perpendicularly oriented collagen fibril layers, i.e. cartilage zones, was measured and the retardation intensity was quantitated (Fig. 3). The results were expressed as area-integrated retardation values (AIR, nm/pixel). A single pixel area was 2.01 μm^2. If partial fibrillation of the superficial zone was seen, the retardation peak coming from the surface (S1–S3, see Fig. 3) was not divided into the three subclasses but the signal intensity was expressed only in class S3.

SAFRANIN-O STAINING OF GAGS AND DIGITAL DENSITOMETRY

The extracellular GAGs were stained with safranin-O as previously described[33]. Light absorbed by the stain was quantitated under monochromatic light with Leitz Ortholux II microscope and Fluotar 25×/0.60 N.A. objective. An interference filter (492 nm±1%, Spindler & Hoyer, Goettingen, Germany) was inserted into the light path to enhance light absorption due to safranin-O[36]. The background image without a specimen containing possible optical defects and readout noise was registered. A neutral density filter set (Schott Glaswerke, Wiesbaden, Germany) ranging from 0 to 3.6 absorbance units was used for the density calibration of the same camera and image-analysis system as used for polarized light microscopy. The correlation coefficient for the camera gray response to the density change between 0 and 3.6 absorbance units was excellent (R^2=0.996) when the background image was subtracted from the image of interest. After calibration, gray values of the background subtracted real images were converted to absorbance units.

From several series of histological sections, two were chosen at random for safranin-O densitometry. Because the location of the tidemark was sometimes difficult to discern, the distribution of the stain was measured from the articular cartilage surface to the osteochondral junction (Fig. 3). A 318-μm wide region of interest, perpendicular to the surface, was chosen from the central condylar area, and the coordinates of the cartilage surface as well as the osteochondral junction were registered. The *mode* absorbance value was calculated along each horizontal pixel row between the marked coordinates. The mode absorbance was adopted since it is insensitive to transmission of light through cell lacunae or other focal, optical artifacts. Finally, the mode absorbance profile was divided into six fractions of equal thickness. In case of partial superficial zone OA was observed, no data was included in the most superficial zone. For each zone of an animal, pixel integrated optical density was calculated from the two measured sections.

STATISTICAL METHODS

Statistical analysis was carried out using SPSS software for Macintosh (SPSS Inc., Chicago, IL, U.S.A.). The prevalence of OA in the young and adult groups was tested with Pearson's Chi-Square test. Mann–Whitney *U* test was utilized for the testing of body weight, cartilage thickness, safranin-O and polarized light microscopy data of the runner and the control groups.

Results

The guinea-pigs were fully grown at the age of 28 weeks when the mean body weight of runners had increased from 577±39 g (at 10 weeks) to 1023±53 g (Fig. 1). The 3+15-week treadmill running did not change the body weight of

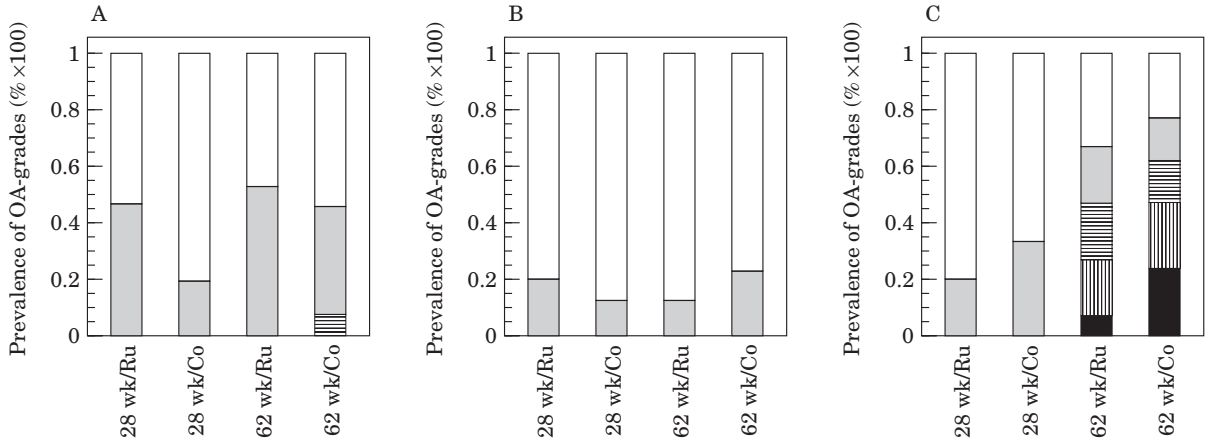

Fig. 4. The prevalence (%) and grading of OA on the WB (A) and NWB (B) areas of femur and weightbearing tibia (TIBIA; C) at 10, 28, 44 and 62 weeks of age in runner (Ru) and control (Co) animals, respectively. Grades: 0: intact articular cartilage (□); 1: superficial lesions not affecting the intermediate zone (▤); 2: lesions extending to the intermediate zone (▤); 3 (▥) and 4 (■): lesions reaching the deep cartilage and subchondral bone, respectively. No significant differences were observed between the groups, Pearson's Chi-square test. For abbreviations, see legend for Fig. 2.

the animals nor affected the cartilage thickness in either experimental group. In controls as a whole, there was a slight tendency towards thinning of articular cartilage with advancing age but this finding was not statistically significant.

Aging, but not running training, increased the prevalence and the severity of osteoarthritic lesions in the guinea-pigs, especially in the tibia (Fig. 4). Young 28-week-old runners exhibited more local superficial fibrillation (grade I OA) than the age-matched controls, but the difference was not statistically significant (Fig. 4). The WB area of the femur exhibited mild age-associated and progressive degeneration of superficial articular cartilage. The degenerative changes were mild in the NWB area, their prevalence was small and they did not progress with age (Fig. 4). Loadbearing tibia was affected most frequently and severely.

In the femurs of young growing, 28-week-old runners, quantitative PLM revealed an increase, up to 24%, in the optical retardation (AIR) by the superficial articular cartilage collagen fibrils both in the WB and NWB areas (Fig. 5). However, in 62-week-old runners the response was entirely opposite, and lower AIR values, being even as low as 51% of the the AIR values of controls, were observed (P<0.01–0.001) (Fig. 5). The collagen network in deep articular cartilage (D1–D3) was markedly less sensitive to joint loading. In TIBIA, the superficial collagen network showed increased birefringence in the 28-week-old guinea-pigs, too. Interestingly, also those few (N=5) 62-week-old guinea-pigs which had an intact superficial zone instead of the fibrillated or fragmented one (N=8), demonstrated increased superficial birefringence after running training (Fig. 5).

Running exercise affected only slightly the absolute thicknesses of the collagen fibril layers located parallel, obliquely, or perpendicular to the articular cartilage, i.e. the thicknesses of the superficial, intermediate, and deep cartilage zones (Table I). The superficial zone of femur proved to be significantly thicker than this zone in tibia (Table I, Fig. 5).

In the young growing or adult mature guinea-pigs, no significant differences were found in the GAG contents or distribution measured with safranin-O after 3+15-week treadmill running (Fig. 6). The response was similar in the WB, NWB and TIBIA areas. There was an overall increase in the GAG-content in the two femoral locations with age, but running did not significantly modulate this property, even though the runners exhibited a slight reduction of GAGs in the superficial cartilage in NWB. The GAG content was also lowest in the NWB area (Fig. 6). It is important to note that the GAG contents represent situations in non-destructed cartilage where loss of tissue has not occurred.

Discussion

Running on the treadmill 2500 m/day, 5 days a week, for 15 weeks at a speed of 0.33 m/s did not have any influence on body weight either in the young growing 28-week-old or adult mature 62-week-old guinea-pigs. At 10 weeks, articular cartilage had almost reached its full thickness. At this time point, however, body weight of the guinea-pigs was only about 60% of that at the age of 28 weeks.

The first signs of partial degradation of the superficial zone of articular cartilage were detected in a few animals at the early age of 10 weeks. With aging, the WB and TIBIA demonstrated an increase in the prevalence and severity of degeneration in control animals. Running training did not affect the incidence or severity of visible OA defects even in the older runners. It is possible that if the cartilage specimens were collected later, i.e. 3–4 months after running training, it might have been possible to record differences in the OA prevalence and severity between the older runners and controls. If this is the case it remains to be shown in future. In the femur, the low OA prevalence bears resemblance to an earlier finding where OA lesions showed little progress with age[30]. At the age of 62 weeks, most lesions were mild and affected only partially the superficial zone (grade I OA; in NWB). In WB and TIBIA, however, the lesions affected also the intermediate and deep zones. This finding appears to emphasize the importance of local mechanical factors in governing the catabolic response of the articular cartilage, i.e. mild structural defects progress towards OA only if they are exposed to mechanical stress. In TIBIA, the superficial zone of tangentially oriented collagen fibrils was thinner than in WB and NWB and showed

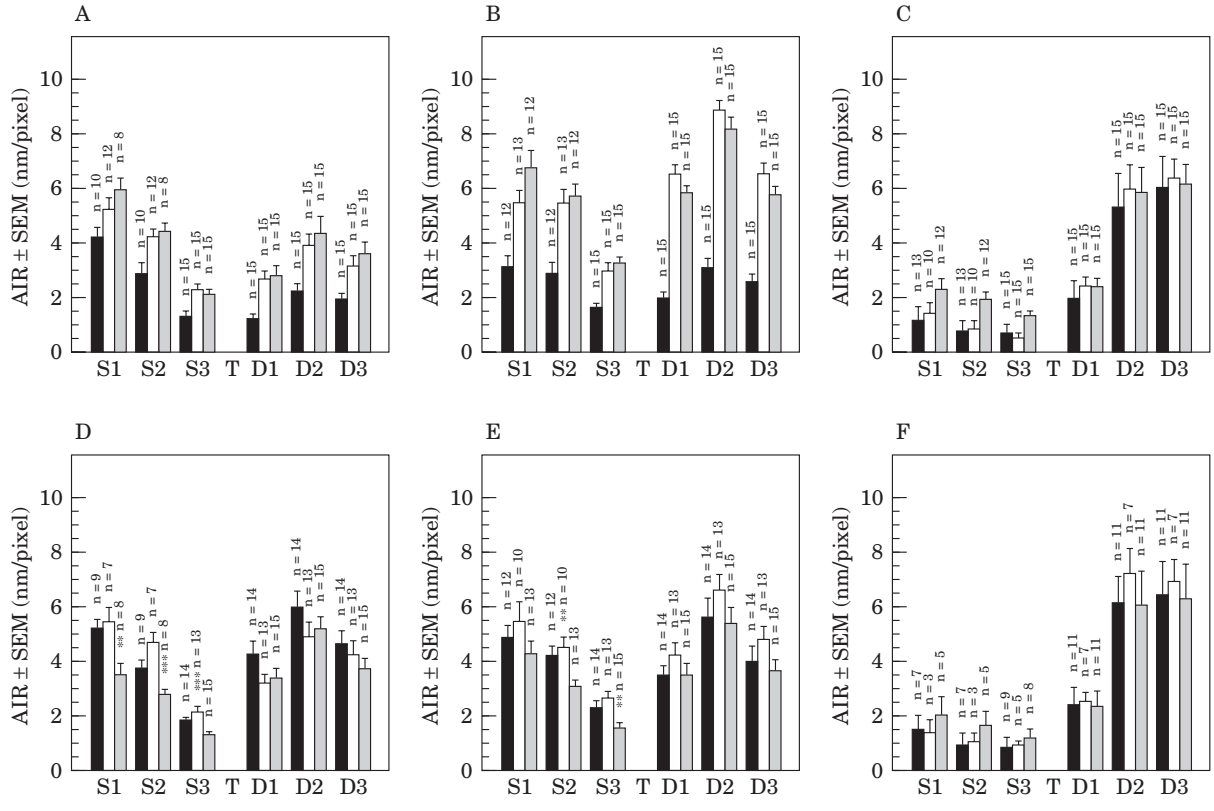

Fig. 5. Optical retardation of polarized light by articular cartilage collagen in the WB (A,D), NWB (B,E) and TIBIA (C,F) locations after 15-week running exercise. Data are expressed as area integrated retardation (AIR) (nm/pixel). For abbreviations, see legend for Fig. 2. Runners and age-matched controls were compared with Mann–Whitney U statistics. **, $P<0.01$; ***, $P<0.001$. A–C: controls at 10 weeks of age, ■; controls at 28 weeks of age, □; runners at 28 weeks of age, ▨. D–F: controls at 44 weeks of age, ■; controls at 62 weeks of age, □; runners at 62 weeks of age, ▨.

lower birefringence (Table I, Fig. 5). This can be an explanation why OA frequency was high in TIBIA.

Our earlier observations from dogs after running training for 15 weeks (40 km/day, 5 days a week)[35], osteotomy of the tibia that causes early OA of the knee[37] or digestion *in vitro* of the collagen network in cartilage explants by collagenase[38], have all shown the reduction of collagen-induced birefringence, especially in the superficial zone. This decrease of birefringence could be detected prior to evident cartilage fibrillation or injury. It is also noteworthy that mice heterozygous for the knock-out of type II procollagen gene (Col2a1+/− mice) show reduced articular cartilage birefringence, softer cartilage, and more OA than the controls[39,40]. Thus, the reduced collagen-induced birefringence observed in the adult mature guinea-pigs after running training strongly suggests a weakened articular cartilage.

The superficial articular cartilage collagen network was surprisingly sensitive to running training when it was analysed with the quantitative PLM (Fig. 5). In the young growing and the adult mature guinea-pigs, two entirely different responses of collagen network to running training were observed. During rapid growth and weight gain, between 10 and 28 weeks, running clearly enhanced the optical retardation values in the superficial collagen layer. The finding was similar in the WB, NWB and TIBIA. This response represented most probably an anabolic structural adaptation of articular cartilage to increased functional demands, and it probably led to strengthening of the most superficial tissue. By contrast, in the adult mature runners, the significantly decreased AIR values of the superfical collagen fibrils at WB and NWB areas probably presented the earliest initial stage of disorganization and/or mechanical injury of the collagen network of cartilage. We do not know whether the decreased optical retardation of the superficial collagen fibrils was due to selective local collagenolytic activity in the cartilage by metalloproteinases or direct mechanical trauma, or both.

The quantitative PLM data indicate that even though adult articular cartilage collagen network has been regarded very stable, especially on account of slow turnover[5], joint loading was able to modulate its properties profoundly. The gradually increasing optical retardation values of cartilage collagen, at least up to the age of 28 weeks, implies that the establishment of the collagen network continues until the individual gains full body weight and musculoskeletal maturity. Similar observations have been made regarding maturation of the human articular cartilage[41]. In humans, the maturation of the collagen network starts from the surface of articular cartilage and progresses towards the subchondral bone during the second decade of life[41]. After reaching the musculoskeletal maturity, i.e. when the cartilage zones are established, articular cartilage seems to become less ready to adapt to, and resist, major variations of mechanical stress[41]. This view was corroborated here by the observation that the collagen network of adult mature guinea-pigs showed significant decline of birefringence after running training.

The birefringence of polarized light by collagen is affected both by the content and organization of the fibrils

Table I
The thickness of collagen fibril zones oriented tangentially, obliquely and perpendicularly to the cartilage surface in 28- and 62-week-old guinea-pigs

	Zone thickness*			
	Tangential	Oblique	Perpendicular	
WB				
Controls, 10 weeks	47.6±3.5	9.38±1.4	117.3±9.3	
Runners, 28 weeks	30.1±2.0	11.9±0.9	100.4±7.8	
Controls, 28 weeks	29.6±3.0	12.6±1.4	103.0±5.7	
Runners, 62 weeks	16.7±1.5	12.4±0.6	142.8±9.3	†P=0.01
Controls, 62 weeks	21.8±3.0	11.9±1.62	121.3±8.7	
NWB				
Controls, 10 weeks	32.8±2.8	19.7±4.7	106.5±9.3	
Runners, 28 weeks	34.6±2.6	14.4±1.37	165.3±4.8	†P=0.05
Controls, 28 weeks	33.8±1.2	15.8±1.6	184.3±7.7	
Runners, 62 weeks	30.7±3.2	18.1±2.3	159.7±10.9	
Controls, 62 weeks	33.2±3.3	15.3±1.6	169.2±12.4	
TIBIA				
Controls, 10 weeks	4.5±3.0	29.9±9.3	68.2±16.8	
Runners, 28 weeks	9.4±3.3	31.9±11.7	68.0±19.3	
Controls, 28 weeks	11.1±1.8	18.6±4.2	88.4±6.2	
Runners, 62 weeks	9.2±4.9	29.0±3.6	67.8±14.3	
Controls, 62 weeks	9.6±3.0	29.9±4.1	71.3±7.0	

*Division to tangential, oblique and perpendicular zones was made according to the preferential course of collagen fibrils determined by quantitative polarized microscopy. Thickness μm±S.E.M.

WB=weightbearing area of medial femur, NWB=non-weightbearing area of medial femur, TIBIA=medial, weightbearing tibia uncovered by meniscus.

†Runner–control comparison between animals of the same age, Mann–Whitney U statistics.

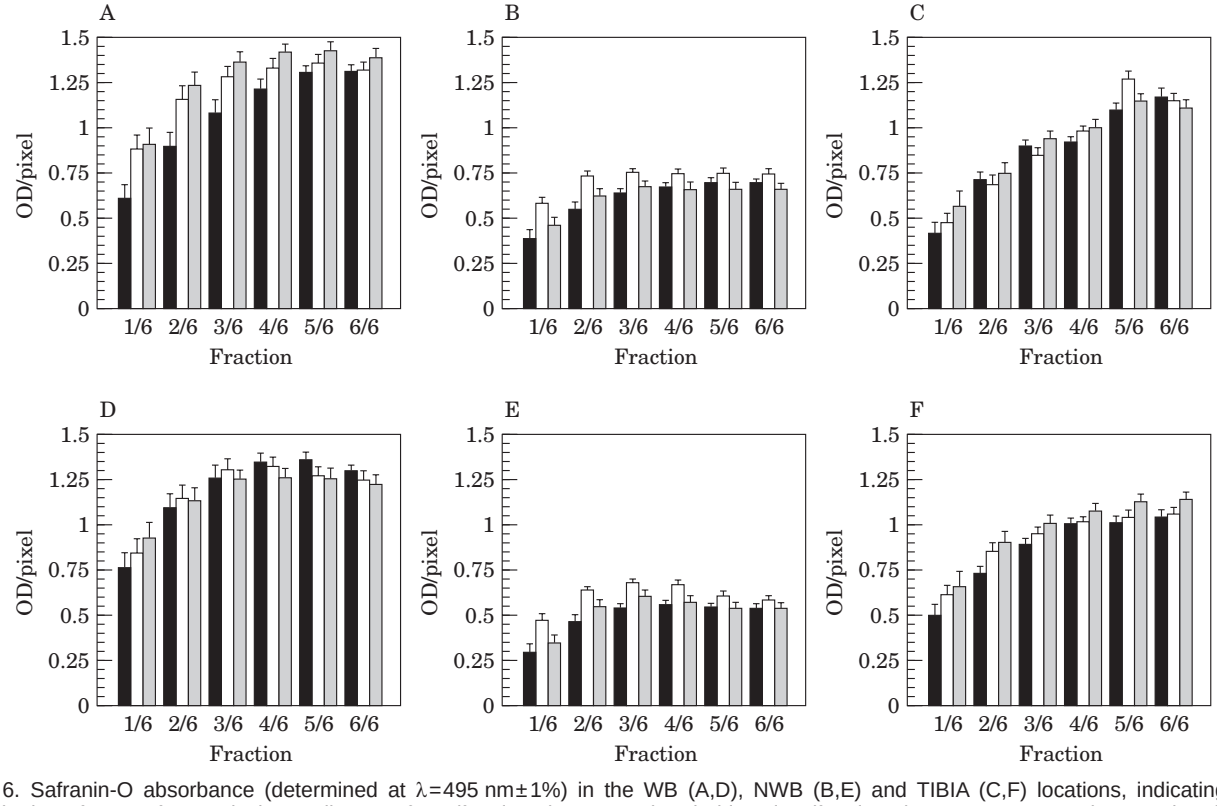

Fig. 6. Safranin-O absorbance (determined at λ=495 nm±1%) in the WB (A,D), NWB (B,E) and TIBIA (C,F) locations, indicating the distribution of GAGs from articular cartilage surface (fraction 1) to osteochondral junction (fraction 6), at 10, 28, 44, and 62 weeks of age. Absorbance is expressed as area-integrated optical density (OD/pixel). No significant differences between runners and age-matched controls in Mann–Whitney U test. A–C: controls at 10 (■) and 28 (□) weeks of age; runners at 28 weeks of age, ▨. D–F: controls at 44 (■) and 62 (□) weeks of age; runners at 62 weeks of age, ▨.

within the matrix. Using EM stereology, biochemical methods, and PLM to reveal the effects of collagenase digestion on collagen fibrils in articular cartilage explants *in vitro*, we have observed that even after a significant decrease of volume fraction and mass of collagen, collagen birefringence can locally remain unchanged if the organization pattern of fibrils do not change, or change favourably as regards to fibril orientation (Langsjo *et al*., manuscript under revision). Therefore, we assume that in this study the reduced birefringence primarily represented disorganization of the collagen fibril network. This hypothesis is also supported by the fact that the superficial collagen layer thickness did not change significantly.

After running, the young growing (28-week-old) and the adult mature (62-week-old) guinea-pigs exhibited an almost unchanged GAG content in the normal-looking articular cartilage, even though the upper third of the cartilage demonstrated slight GAG increase both in WB and TIBIA (Fig. 6). The finding is very similar to that observed in surgically induced early OA of guinea-pigs[32]. It is possible, even probable, that running training affected the synthesis and turnover of cartilage proteoglycans. Earlier, we have demonstrated enhanced GAG metabolism with increased concentration of GAGs in cartilage of young dogs after short-term running training[42]. On the other hand, the idea of accelerated metabolism of the superficial chondrocytes in the adult mature runners can be linked to slightly increased synthesis coupled with enhanced degradation of aggrecans to smaller fragments residing in the matrix[4,43,44]. In this study, GAGs were not determined from fibrillated or injured articular cartilage.

The data from PLM and safranin-O staining, at 10, 28, 44 and 62 weeks of age, showed a gradual, age-associated modulation of collagen network and slight increase of GAG content, which took place especially in the upper third of articular cartilage. This can be interpreted to represent an effort by the tissue to reach a higher level of resistance against mechanical stresses and shear experienced by increased body weight and loading.

In summary, similar kind of physiological joint loading could either promote or deteriorate the formation and/or organization of the fibrillar collagens of articular cartilage, especially in the superficial zone of the tissue. The tissue response depended on the age of the guinea-pigs. In young adult animals, running training increased the collagen-induced birefringence of articular cartilage indicating either improved orientation of collagen fibrils and/or increased amount of collagen. This probably strengthened the cartilage. In adult mature guinea-pigs, however, the significantly reduced birefringence after similar running training wittnessed of an untoward response of collagen network to joint loading. With time, the weaker collagen network probably predisposes the articular cartilage to early OA.

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